Swati Haldar (2010). ***Multiple Functionalities of chromodomain protein Swi6 in Heterochromatin Structure and Function in Fission Yeast.*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.
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SUMMARY OF THE THESIS

Summary Points:

- **Mutations** in the conserved residues of the CUE motif of Uhp1 affect its interaction with Ubiquitin *in vitro*. However, the scenario is exactly opposite *in vivo* where, one of these mutations (P42A) has been found to improve its interaction with Ubiquitin.
- Uhp1 affects silencing at *otr* region in both Rhp6-dependent and independent manner; at *imr*, the effect is entirely independent of Rhp6.
- Uhp1 may act at an interface of heterochromatin and euchromatin and helps to determine the direction to which the dynamic equilibrium is to be shifted.
- Uhp1 favours the formation of heterochromatin. It appears to facilitate demethylating H3K4 on one hand, while preventing any further H3K4 dimethylation by sequestering SET1. Its interaction with Clr4 may facilitate H3K9 dimethylation, thus, gearing up in the direction of heterochromatin formation. However, Uhp1 does not interact directly with Swi6 although, it affects the recruitment of latter, the effect of Swi6 recruitment may be indirect one resulting from lack of Clr4 recruitment and H3K9 methylation in *uhp1Δ* mutant.

IV.5.4. DISCUSSION

Results from previous and present study highlight three features of Uhp1: (i) Uhp1 is a ubiquitylation target of Rhp6 (ii) Uhp1 has a FMN binding domain (iii) Uhp1 has CUE motif needed for ubiquitin binding. These features, in turn, contribute to three different modes of action of Uhp1 in silencing in fission yeast. Each of these aspects are summarised below briefly:

IV.5.4.1. Uhp1 in the Rhp6-dependent pathway

Evidences in support of this aspect are largely the outcomes of previous studies. Rhp6 (the *S. cerevisiae* RAD6 homologue in *S. pombe*) plays a role in switching dependent silencing at mating type locus in *S. pombe* (Singh *et al.*, 1998) and so, did Uhp1 and identified as the target/mediator of Rhp6 (Naresh *et al.*, 2003). Rhp6 polyubiquitinated Uhp1 and this modification is correlated with the nuclear localization of Uhp1 during S-phase (Dr. Sharanjot Saini, Ph.D. Thesis, 2005). Current results show that Uhp1 also affects silencing at *otr* region of centromere in Rhp6-dependent manner.

Histone H3K4 methylation in *S. cerevisiae* is mediated by Set1 complex (Set1C) and is dependent upon H2B ubiquitylation by RAD6. Protein machinery for H3K4 methylation, its relationship to RAD6/Rhp6 regulation and enzymology is highly conserved between budding and fission yeasts (Roguev *et al.*, 2003). Earlier results showed that Rhp6 monoubiquitinated H2B. However, this reaction was found to be independent of Uhp1 (Dr. Sharanjot Saini, Ph.D. Thesis, 2000).

IV.5.4.2. Is Uhp1 a part of the conserved RAD6/Set1 pathway of H3K4 dimethylation?

Elevated levels of H3K4 dimethylation in *uhp1Δ* mutant cells clearly indicated that Uhp1 plays a role in negatively regulating H3K4 dimethylation in *S. pombe*. Further, experiments showed that Uhp1 not only interacted with Set1 but also possesses H3K4 demethylase activity, which could explain the above observation.

H3K4 methylation via RAD6/Rhp6 dependent ubiquitylation of H2B is conserved in both budding and fission yeasts (Roguev *et al.*, 2003). Ubiquitylation of H2B by Rhp6 is independent of Uhp1 (Ph.D. Thesis, Dr. Sharanjot Saini, 2005). This apparently keeps Uhp1 out of the conserved RAD6/Set1 H3K4 methylation pathway. BLAST search shows a few putative homologues with high sequence homology to Uhp1 in *S. cerevisiae*. Further, study will be needed to establish which one of these is the functional homologue of Uhp1.

However, our observations that the level of me_2H3K4 is increased in *uhp1Δ* mutant and that Uhp1 interacted with Set1 *in vivo* suggest that Uhp1 introduces an element of direct negative regulation into this pathway in *S. pombe* (the scenario awaits investigation in *S. cerevisiae*). Uhp1 exerts the positive control on me_2H3K9 which may be correlated with its interaction with Clr4 and reduced me_2H3K9 in *uhp1Δ* mutant. Thus, Uhp1 may act as a switch turning the equilibrium of the chromatin dynamics from euchromatin towards heterochromatin.

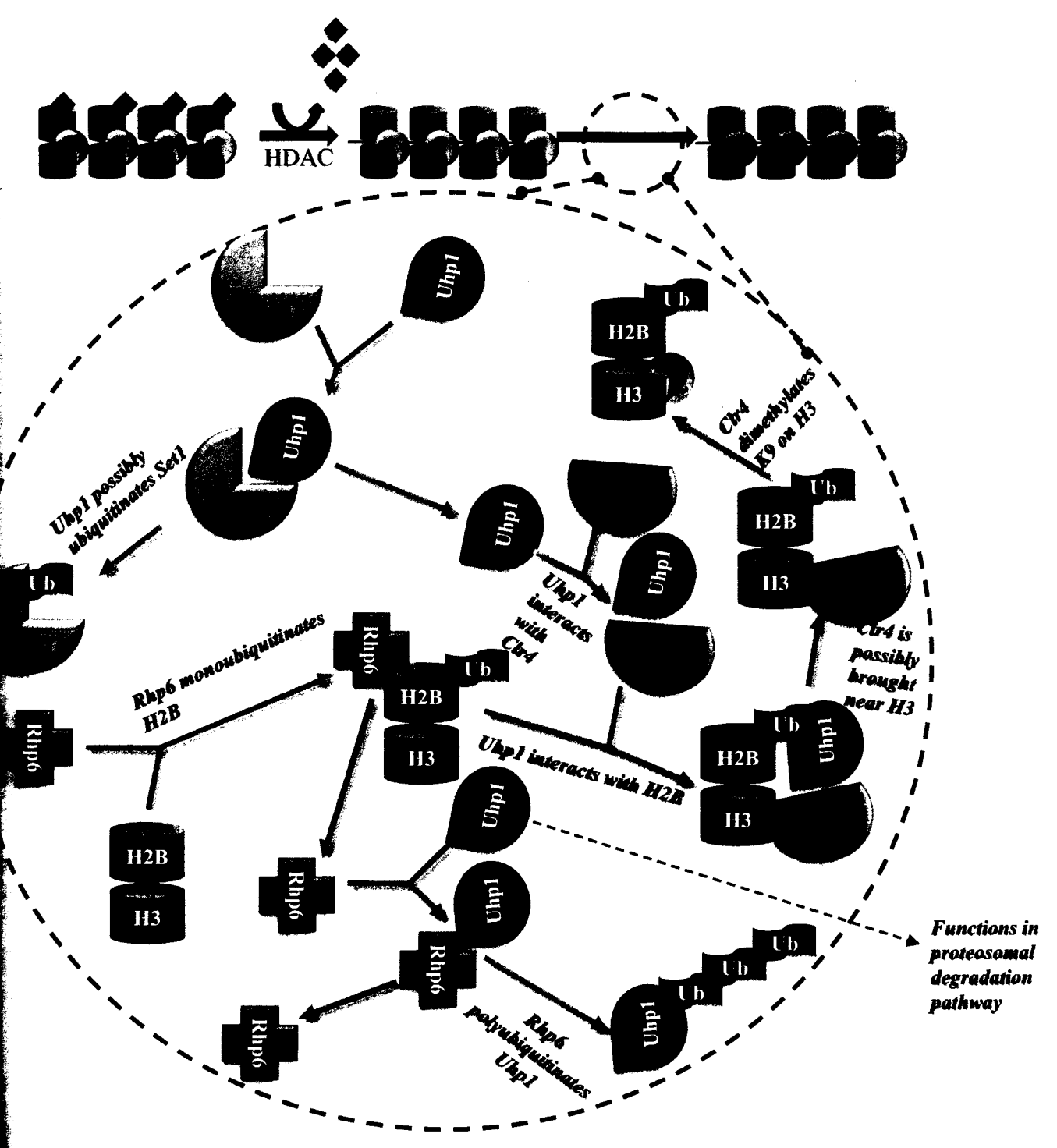
So the answer to the above question is that Uhp1 is a part of the H3K4 dimethylation pathway, albeit as a negative regulator.

IV.5.4.3. CUE motif of Uhp1 functionally places it in a slightly different category

Rhp6 polyubiquitylates Uhp1, a covalent modification. Moreover, Uhp1 binds single ubiquitin moiety in a non-covalent manner (Ph.D. thesis, Dr. Sharanjot Saini, 2005). Our results showed that the conserved CUE motif (known for ubiquitin binding) in Uhp1 influences the mono-ubiquitin binding *in vitro*.

Ubiquitin binding due to CUE motif identifies a function of Uhp1, which is independent of Rhp6. Furthermore, observations like occurrence of multi-septate cells together with lagging mitotic chromosomes (attributes of improperly controlled Septation Initiation Network or SIN pathway) in CUE motif mutants of Uhp1 indicate that mono-ubiquitin binding of Uhp1 somehow controls this pathway. Cellular levels of certain factors like Byr4 need to be controlled for proper activation of SIN pathway. Byr4 is degraded via proteosomal pathway and is dependent on Cdc16 (Krapp *et al.*, 2008). Cdc16 was shown long ago to affect septation and *cdc16-116* mutant formed multi-septate cells (Minet *et al.*, 1979). Besides, interaction with DphI (one of the three ubiquitin binding proteins in *S. pombe* that protect multiubiquitinated proteins from de-ubiquitylation, unpublished data of Gordon *et al.*, personal communication) strongly supports Uhp1 having a role in protein turn-over pathway.

Based on the observations, we prepare a speculative working model for the role of Uhp1 in silencing (Fig.IV.5.23), according to which, Rhp6 mono-ubiquitylates histone H2B, to which Uhp1 binds. Physical interaction between Uhp1 and Clr4 serves to bring latter into the vicinity of histone H3. This close proximity is likely to facilitate the dimethylation of K9 on H3. In parallel to this, the demethylase activity of Uhp1 helps to remove the me_2 group from K4 on H3 and tilt the equilibrium towards heterochromatin formation. On the other hand the interaction between Uhp1 and SET1


HDAC
Uhp1
Ub
H2B
H3
Ctr4 dimethylates K9 on H3
Uhp1 possibly ubiquitinates Set1
Uhp1 interacts with Ctr4
Ctr4 is possibly brought near H3
Rhp6 monoubiquitinates H2B
Rhp6
Uhp1 interacts with H2B
Functions in proteosomal degradation pathway
Rhp6 polyubiquitinates Uhp1

may block latter from dimethylating K4 on H3. Furthermore, in a not so related pathway, Uhp1 interacts with DphI, binds mono-ubiquitin, possibly prevents deubiquitination of proteosomal targets and in turn, exerts a global effect on cell physiology.